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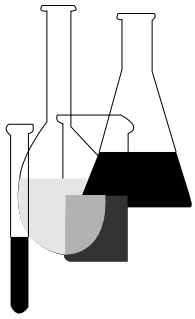
Health Effects Test Guidelines OPPTS 870.7485 Metabolism and Pharmacokinetics



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.7485 Metabolism and pharmacokinetics

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are 40 CFR 798.7485 Metabolism and pharmacokinetics; OPP 85–1 Metabolism Study (Pesticide Assessment Guidelines, Subdivision F - Hazard Evaluation: Human and Domestic Animals, EPA report 540/09-82-025, October 1982); and OECD Guideline 417 Toxicokinetics.

(b) **Purpose.** (1) Testing of the disposition of a test substance is designed to obtain adequate information on its absorption, distribution, biotransformation, and excretion and to aid in understanding the mechanism of toxicity. Basic pharmacokinetic parameters determined from these studies will also provide information on the potential for accumulation of the test substance in tissues and/or organs and the potential for induction of biotransformation as a result of exposure to the test substance. These data can be used to assess the adequacy and relevance of the extrapolation of animal toxicity data (particularly chronic toxicity and/or carcinogenicity data) to human risk assessment.

(2) Metabolism data can also be used to assist in determining whether animal toxicity studies have adequately addressed any toxicity concerns arising from exposure to plant metabolites, and in the setting of tolerances, if any, for those metabolites in raw agricultural commodities.

(c) **Definitions.** The following definitions apply to this guideline:

Metabolism (biotransformation) is the sum of the processes by which a foreign chemical is subjected to chemical change by living organisms.

LOEL is the lowest observable effect level.

NOEL is the no observable effect level.

Pharmacokinetics is the quantitation and determination of the time course and dose dependency of the absorption, distribution, biotransformation, and excretion of chemicals.

(d) **Good laboratory practice standards.** The pharmacokinetics and metabolism tests outlined in this guideline are to conform to the laboratory practices stipulated in 40 CFR parts 160 and 792 (Good Laboratory Practice Standards).

(e) **Test Procedures.** Test procedures presented below utilize a tier system to minimize the use of resources and to allow flexibility in the conduct of metabolism studies. The proposed tier system will consist of

a basic data set (Tier 1) and additional studies (Tier 2) which may be requested based upon the existing toxicology data base and/or the results of Tier 1 testing which are found to impact upon the risk assessment process. For Tier 1 testing, the oral route will typically be required; however, if the use pattern results in other types of exposure, other routes (dermal and/or inhalation) may be required for initial testing of the disposition of a chemical substance. The registrant should justify the route of exposure to the Agency. Complete descriptions of the test procedures for these other routes of exposure can be found under paragraph (i) of this guideline. Except in unusual circumstances, the tiered approach to metabolism testing should apply to all listed routes of exposure.

(1) **Pilot studies.** The use of pilot studies is recommended and encouraged for the selection of experimental conditions for the pharmacokinetics and metabolism studies (mass balance, analytical procedures, dose-finding, excretion of CO_2, etc.).

(2) **Animal selection**—(i) **Species.** The rat shall normally be used for testing because it has been used extensively for metabolic and toxicological studies. The use of other or additional species may be required if critical toxicology studies demonstrate evidence of significant toxicity in these species or if metabolism is shown to be more relevant to humans in the test species.

(ii) **Strain.** Adult animals of the strain used or proposed to be used for the determination of adverse health effects associated with the test substance.

(3) **Material to be tested**—(i) **Test substance.** (A) A radiolabeled test substance using ^{14}C should be used for all material balance and metabolite identification aspects of the study. Other radioactive and stable isotopes may be used, particularly if the element is responsible for or is a part of the toxic portion of the compound. If it can be demonstrated that the material balance and metabolite identification requirements can be met using unlabeled test substance, then radiolabeled compound need not be used. If possible, the radiolabel should be located in a core portion of the molecule which is metabolically stable (it is not exchangeable, is not removed metabolically as CO_2, and does not become part of the one-carbon pool of the organism). Labeling of multiple sites of the molecule may be necessary to follow the metabolic fate of the compound.

(B) The label should follow the test compound and/or its major metabolites until excreted. The radiopurity of the radioactive test substance shall be the highest attainable for a particular test substance (ideally it should be greater than 95 percent) and reasonable effort should be made to identify impurities present at or above 2 percent. The purity, along with the identity of major impurities which have been identified, shall be reported. For other segments of the study, nonradioactive test substance may

be used if it can be demonstrated that the analytical specificity and sensitivity of the method used with nonradioactive test substance is equal to or greater than that which could be obtained with the radiolabeled test substance. The radioactive and nonradioactive test substances shall be analyzed using an appropriate method to establish purity and identity. Additional guidance will be provided in chemical specific test rules to assist in the definition and specifications of test substances composed of mixtures and methods for determination of purity.

(ii) **Administration of test substance.** Test substance should be dissolved or suspended homogeneously in a vehicle usually employed for acute administration. A rationale for the choice of vehicle should be provided. The customary method of administration will be by oral gavage; however, administration by gelatin capsule or as a dietary mixture may be advantageous in specific situations. Verification of the actual dose administered to each animal should be provided.

(4) **Tier testing.** (i) The multiplicity of metabolic parameters that impact the outcome of toxicological evaluations preclude the use of a universal study design for routine toxicological evaluation of a test substance. The usefulness of a particular study design depends upon the biological activity of a compound and circumstances of exposure. For these reasons, a tiered system is proposed for evaluation of the metabolism/kinetic properties of a test substance.

(ii) The first tier data set is a definitive study by the appropriate route of exposure conducted in male rats to determine the routes and rate of excretion and to identify excreted metabolites. First tier data will also provide basic information for additional testing (Tier 2) if such testing is considered necessary. In the majority of cases, Tier 1 data are expected to satisfy regulatory requirements for biotransformation and pharmacokinetic data on test chemicals.

(iii) Second tier testing describes a variety of metabolism/kinetic experiments which address specific questions based on the existing toxicology data base and/or those results of Tier 1 testing impacting significantly on the risk assessment process. For conduct of these studies, individualized protocols may be necessary. Protocols for these studies, if required, can be developed as a cooperative effort between Agency and industry scientists.

(f) **Tier 1 data requirements (minimum data set).** At this initial level of testing, biotransformation and pharmacokinetic data from a single low dose group will be required. This study will determine the rate and routes of excretion and the type of metabolites generated.

(1) **Number and sex of animals.** A minimum of four male young adult animals will be required for Tier 1 testing. The use of both sexes

may be required in cases where there is evidence to support significant sex-related differences in toxicity.

(2) **Dose selection.** (i) A single dose is required for each route of exposure. The dose should be nontoxic, but high enough to allow for metabolite identification in excreta. If no other toxicity data are available for selection of the low dose, a dose identified as a fraction of the LD50 (as determined from acute toxicity studies) may be used. The magnitude of the dose used in Tier 1 studies should be justified in the final report.

(ii) For test substances of low toxicity a maximum dose of 1,000 mg/kg should be used; chemical-specific considerations may necessitate a higher maximum dose and will be addressed in specific test rules.

(3) **Measurements**—(i) **Excretion.** (A) Data obtained from this section (percent recovery of administered dose from urine, feces, and expired air) will be used to determine the rate and extent of excretion of test chemical, to assist in establishing mass balance, and will be used in conjunction with pharmacokinetic parameters to determine the extent of absorption. The quantities of radioactivity eliminated in the urine, feces, and expired air shall be determined separately at appropriate time intervals.

(B) If a pilot study has shown that no significant amount of radioactivity is excreted in expired air, then expired air need not be collected in the definitive study.

(C) Each animal is to be placed in a separate metabolic unit for collection of excreta (urine, feces and expired air). At the end of each collection period, the metabolic units are to be rinsed with appropriate solvent to ensure maximum recovery of radiolabel. Excreta collection shall be terminated at 7 days, or after at least 90 percent of the administered dose has been recovered, whichever occurs first. The total quantities of radioactivity in urine are to be determined at 6, 12, and 24 h on day 1 of collection, and daily thereafter until study termination, unless pilot studies suggest alternate or additional time points for collection. The total quantities of radioactivity in feces should be determined on a daily basis beginning at 24 h post-dose, and daily thereafter until study termination. The collection of CO_2 and other volatile materials may be discontinued when less than 1 percent of the administered dose is found in the exhaled air during a 24-h collection period.

(ii) **Tissue distribution.** At the termination of the Tier 1 study, the following tissues should be collected and stored frozen: Liver, fat, gastrointestinal tract, kidney, spleen, whole blood, and residual carcass. If it is determined that a significant amount of the administered dose is unaccounted for in the excreta, then data on the percent of the total (free and bound) radioactive dose in these tissues as well as residual carcass will be requested. Additional tissues shall be included if there is evidence of target organ toxicity from subchronic or chronic toxicity studies. For other

routes of exposure, specific tissues may also be required, such as lungs in inhalation studies and skin in dermal studies. Certain techniques currently at various stages of development, e.g. quantitative whole-body autoradiography, may prove useful in determining if a test substance concentrates in certain organs or in determining a specific pattern of distribution within a given tissue. The use of such techniques is encouraged, but not required, and may be employed to limit the number of tissues collected to those shown to contain a measurable amount of radioactivity.

(iii) **Metabolism.** Excreta shall be collected for identification and quantitation of unchanged test substance and metabolites as described under paragraph (f)(3)(i) of this guideline. Pooling of excreta to facilitate metabolite identification within a given dose group is acceptable. Profiling of metabolites from each time period is recommended. However, if lack of sample and/or radioactivity precludes this, pooling of urine as well as pooling of feces across several time points is acceptable. Appropriate qualitative and quantitative methods shall be used to assay urine, feces, and expired air from treated animals. Reasonable efforts should be made to identify all metabolites present at 5% or greater of the administered dose and to provide a metabolic scheme for the test chemical. Compounds which have been characterized in excreta as comprising 5 percent or greater of the administered dose should be identified. If identification at this level is not possible, a justification/explanation should be provided in the final report. Identification of metabolites representing less than 5 percent of the administered dose might be requested if such data are needed for risk assessment of the test chemical. Structural confirmation should be provided whenever possible. Validation of the methods used in metabolite identification should be included.

(g) **Tier 2 data requirements.** Studies at the Tier 2 level are designed to answer questions about the disposition of test chemicals based on the existing toxicology data base and/or results of Tier 1 testing which may have a significant impact on the risk assessment for the test chemical. Such studies may address questions regarding absorption, persistence, or distribution of the test chemical, or a definitive alteration in the metabolic profile occurring with dose which may be of toxicological concern. At the Tier 2 level, only those studies which address a specific concern are required, and will be conducted according to mutual agreement between the registrant and the Agency. Flexibility will be allowed in the design of specific experiments as warranted by technological advances in this field.

(1) **Absorption.** (i) If the extent of absorption cannot be established from Tier 1 studies, or where greater than 20 percent of the administered dose is present in feces, a study to determine the extent of absorption will be required. This can be accomplished either through intravenous administration of test material and measurement of radioactivity in excreta

or after oral administration of test material and measurement of radioactivity in bile.

(ii) For the intravenous study, a single dose (not to exceed the oral dose used in Tier 1) of test chemical using an appropriate vehicle should be administered in a suitable volume (e.g. 1 mL/kg) at a suitable site to at least three male rats (both sexes might be used if warranted). The disposition of the test chemical should be monitored for oral dosing as outlined in paragraph (f)(3)(i) of this guideline. Metabolite identification will not be required for this study.

(iii) If a biliary excretion study is chosen the oral route of administration may be requested. In this study, the bile ducts of at least three male rats (or of both sexes, if warranted) should be appropriately cannulated and a single dose of the test chemical should be administered to these rats. Following administration of the test chemical, excretion of radioactivity in bile should be monitored as long as necessary to determine if a significant percentage of the administered dose is excreted via this route.

(2) **Tissue distribution time course.** (i) A time course of tissue distribution in selected tissues may be required to aid in the determination of a possible mode of toxic action. This concern may arise from evidence of extended half-life or possible accumulation of radioactivity in specific tissues. The selection of tissues for this type of study will be based upon available evidence of target organ toxicity and/or carcinogenicity, and the number of time points required will be based upon pharmacokinetic information obtained from Tier 1 data. Flexibility will be allowed in the selection of time points to be studied.

(ii) For this type of study, three rats per time point will be administered an appropriate oral dose of test chemical, and the time course of distribution monitored in selected tissues. Only one sex may be required, unless target organ toxicity is observed in sex-specific organs. Assessment of tissue distribution will be made using appropriate techniques for assessment of total amount distributed to tissue and for assessment of metabolite distribution.

(3) **Plasma kinetics.** The purpose of this experiment is to obtain estimates of basic pharmacokinetic parameters (half-life, volume of distribution, absorption rate constant, area under the curve) for the test substance. Kinetic data may be required if the data can be used to resolve issues about bioavailability and to clarify whether clearance is saturated in a dose-dependent fashion. For this experiment a minimum of three rats per group is required. At least two doses will be required, usually the NOEL and LOEL from the critical toxicology study. Following administration of test substance, samples should be obtained from each animal at suitable time points appropriate sampling methodology. Total radioactivity present (or total amount of chemical, for nonradioactive materials) should be ana-

lyzed in whole blood and plasma using appropriate methods, and the blood/plasma ratio should be calculated.

(4) **Induction.** (i) Studies addressing possible induction of biotransformation may be requested under one or more of the following conditions:

(A) Available evidence indicates a relationship between induced metabolism and enhanced toxicity.

(B) The available toxicity data indicate a nonlinear relationship between dose and metabolism.

(C) The results of Tier 1 metabolite identification studies show identification of a potentially toxic metabolite.

(D) Induction can plausibly be invoked as a factor in such effects where status may depend on the level of inducible enzymes present. Several in vivo and in vitro methods are available for assessment of enzyme induction, and the experiments which best address the issue at hand can be determined between Agency and industry scientists. If induction is demonstrated, the relationship of this phenomenon to toxicity observed from subchronic and/or chronic toxicity studies will need to be addressed.

(iii) If toxicologically significant alterations in the metabolic profile of the test chemical are observed through either in vitro or in vivo experiments, characterization of the enzyme(s) involved (for example, Phase I enzymes such as isozymes of the Cytochrome P450–dependent monooxygenase system, Phase II enzymes such as isozymes of sulfotransferase or uridine diphosphate glucuronosyl transferase, or any other relevant enzymes) may be requested. This information will help establish the relevance of the involved enzyme(s) to human risk, as it is known that certain isozymes are present in animal species which are not present in humans, and vice versa.

(5) **Physiologically-based modeling.** Traditional methods of modeling have been used to determine kinetic parameters associated with drug and xenobiotic disposition, but have assumed a purely mathematical construct of mammalian organisms in their operation. On the other hand, more recent models which take into account the physiological processes of the animal have been used with success in defining biological determinants of chemical disposition as well as the relationship between tissue dose and tissue response. These so-called physiologically-based models, also allow for cross-species extrapolation which is often necessary in the risk-assessment process. The use of physiologically-based modeling as an experimental tool for addressing specific issues related to biotransformation and pharmacokinetics of a test substance is encouraged. Information as derived from physiologically-based modeling experiments may aid in the comparison of biotransformation and pharmacokinetics of a test substance between animal species and humans, and in the assessment of risk under

specific exposure conditions. At the discretion of the Agency, or by mutual agreement, results of physiologically based pharmacokinetic (PBPK) studies with parent compound may be submitted in lieu of other studies, if it is determined that such data would provide adequate information to satisfy this guideline.

(h) **Reporting of study results.** In addition to the reporting requirements specified in 40 CFR part 792, subpart J, the completed study (Tier 1 or Tier 2) should be presented in the following format:

(1) *Title/Cover Page.* Title page and additional requirements (requirements for data submission, good laboratory practice, statements of data confidentiality claims and quality assurance) if relevant to the study report, should precede the content of the study formatted below. These requirements are to be found in 40 CFR parts 158 and 160 or parts 790, 792, and 799.

(2) *Table of Contents.* A concise listing is to precede the body of the report, containing all essential elements of the study and the page and table number where the element is located in the final report of the study. Essential elements of the Table of Contents should include a summary, an introduction, the materials and methods section, results, discussion/ conclusions, references, tables, figures, appendices, and key subsections as deemed appropriate. The Table of Contents should include the page number of each of these elements.

(3) *Body of the report.* The body of the report shall include information required under this guideline, organized into sections and paragraphs as follows:

(i) *Summary.* This section of the study report is to contain a summary and analysis of the test results and a statement of the conclusions drawn from the analysis. This section should highlight the nature and magnitude of metabolites, tissue residue, rate of clearance, bioaccumulation potential, sex differences, etc. The summary should be presented in sufficient detail to permit independent evaluation of the findings.

(ii) *Introduction.* This section of the report should include the objectives of the study, guideline references, regulatory history, if any, and a rationale.

(iii) *Materials and methods.* This section of the report is to include detailed descriptions of all elements including:

(A) *Test substance.* (*1*) This section should include identification of the test substance—chemical name, molecular structure, qualitative and quantitative determination of its chemical composition, and type and quantities of any impurities whenever possible.

(*2*) This section should also include information on physical properties including physical state, color, gross solubility and/or partition coefficient, and stability.

(*3*) The type or description of any vehicle, diluents, suspending agents, and emulsifiers or other materials used in administering the test substance should be stated.

(*4*) If the test substance is radiolabeled, information on the following should be included in this subsection: The type of radionuclide, position of label, specific activity, and radiopurity.

(B) *Test animals.* This section should include information on the test animals, including: Species, strain, age at study initiation, sex, body weight, health status, and animal husbandry.

(C) *Methods.* This subsection should include details of the study design and methodology used. It should include a description of:

(*1*) How the dosing solution was prepared and the type of solvent, if any, used.

(*2*) Number of treatment groups and number of animals per group.

(*3*) Dosage levels and volume.

(*4*) Route of administration.

(*5*) Frequency of dosing.

(*6*) Fasting period (if used).

(*7*) Total radioactivity per animal.

(*8*) Animal handling.

(*9*) Sample collection.

(*10*) Sample handling.

(*11*) Analytical methods used for separation.

(*12*) Quantitation and identification of metabolites.

(*13*) Other experimental measurements and procedures employed (including validation of test methods for metabolite analysis).

(D) *Statistical analysis.* If statistical analysis is used to analyze the study findings, then sufficient information on the method of analysis and the computer program employed should be included so that an independent reviewer/statistician can reevaluate and reconstruct the analysis. Presentation of models should include a full description of the model to allow independent reconstruction and validation of the model.

(iv) *Results.* All data should be summarized and tabulated with appropriate statistical evaluation and placed in the text of this section. Radioactivity counting data should be summarized and presented as appropriate for the study, typically as disintegrations per minute and microgram or milligram equivalents, although other units may be used. Graphic illustrations of the findings, reproduction of representative chromatographic and spectrometric data, and proposed metabolic pathways and molecular structure of metabolites should be included in this section. In addition the following information is to be included in this section if applicable:

(A) Justification for modification of exposure conditions, if applicable.

(B) Justification for selection of dose levels for pharmacokinetic and metabolism studies.

(C) Description of pilot studies used in the experimental design of the pharmacokinetic and metabolism studies, if applicable.

(D) Quantity and percent recovery of radioactivity in urine, feces, and expired air, as appropriate. For dermal studies, include recovery data for treated skin, skin washes, and residual radioactivity in the covering apparatus and metabolic unit as well as results of the dermal washing study.

(E) Tissue distribution reported as percent of administered dose and microgram equivalents per gram of tissue.

(F) Material balance developed from each study involving the assay of body tissues and excreta.

(G) Plasma levels and pharmacokinetic parameters after administration by the relevant routes of exposure.

(H) Rate and extent of absorption of the test substance after administration by the relevant routes of exposure.

(I) Quantities of the test substance and metabolites (reported as percent of the administered dose) collected in excreta.

(J) Individual animal data.

(v) *Discussion and conclusions.* (A) In this section the author(s) should:

(*1*) Provide a plausible explanation of the metabolic pathway for the test chemical.

(*2*) Emphasize species and sex differences whenever possible.

(*3*) Discuss the nature and magnitude of metabolites, rates of clearance, bioaccumulation potential, and level of tissue residues as appropriate.

(B) The author(s) should be able to derive a concise conclusion that can be supported by the findings of the study.

(vi) *Optional sections.* The authors may include additional sections such as appendices, bibliography, tables, etc.

(i) **Alternate routes of exposure for Tier 1 testing**—(1) **Dermal**—(i) **Dermal treatment.** One (or more if needed) dose levels of the test substance shall be used in the dermal portion of the study. The low dose level should be selected in accordance with paragraph (f)(2) of this guideline. The dermal doses shall be dissolved, if necessary, in a suitable vehicle and applied in a volume adequate to deliver the doses. Shortly before testing, fur is to be clipped from the dorsal area of the trunk of the test animals. Shaving may be employed, but it should be carried out approximately 24 h before the test. When clipping or shaving the fur, care should be taken to avoid abrading the skin, which could alter its permeability. Approximately 10 percent of the body surface should be cleared for application of the test substance. With highly toxic substances, the surface area covered may be less than approximately 10 percent, but as much of the area as possible is to be covered with a thin and uniform film. The same nominal treatment surface area shall be used for all dermal test groups. The dosed areas are to be protected with a suitable covering which is secured in place. The animals shall be housed separately.

(ii) **Dermal washing study.** (A) A washing experiment is to be conducted to assess the removal of the applied dose of the test substance by washing the treated skin area with a mild soap and water. A single dose shall be applied to two animals in accordance with paragraph (f)(2) of this guideline. After application (2 to 5 min) the treated areas of the animals shall be washed with a mild soap and water. The amounts of test substance recovered in the washes shall be determined to assess the effectiveness of removal by washing.

(B) Unless precluded by corrosiveness, the test substance shall be applied and kept on the skin for a minimum of 6 h. At the time of removal of the covering, the treated area shall be washed following the procedure as outlined in the dermal washing study. Both the covering and the washes shall be analyzed for residual test substance. At the termination of the studies, each animal shall be sacrificed and the treated skin removed. An appropriate section of treated skin shall be analyzed to determine residual radioactivity.

(2) **Inhalation.** A single (or more if needed) concentration of test substance shall be used in this portion of the study. The concentration should be selected in accordance with paragraph (f)(2) of this guideline. Inhalation treatments are to be conducted using a ‘‘nose-cone’’ or ‘‘head-

only’’ apparatus to prevent absorption by alternate routes of exposure. If other inhalation exposure conditions are proposed for use in a chemical-specific test rule, justification for the modification must be documented. A single exposure over a defined period shall be used for each group—a typical exposure is 4–6 h.